Claims

1. Method of transferring nucleic acid into one or more striated muscles in vivo in which the muscle cells are brought into contact with the nucleic acid to be transferred by direct administration into the tissue or by topical or systemic administration and in which the transfer is brought about by application to the said muscle of one or more electrical pulses of an intensity of between 1 and 800 volts/cm.

2. Method according to claim 1, characterized in that the intensity of the field is between 4 and 400 volts/cm.

3. Method according to claim 1, characterized in that the intensity of the field is between 30 and 300 volts/cm.

4. Method according to one of claims 1 to 3, characterized in that the total duration of application of the electric field is greater than 10 milliseconds.

5. Method according to one of claims 1 to 4, characterized in that the application, to the muscle, of the electric field comprises one or more pulses of regular frequency.

6. Method according to claim 5, characterized in that the application, to the muscle, of the electric field comprises between 1 and 100,000 pulses of frequency between 0.1 and 1000 hertz.

7. Method according to one of claims 1 to

4, characterized in that the electrical pulses are delivered in an irregular manner relative to each other and in that the function describing the intensity of the electric field as a function of time for one pulse is variable.

8. Method according to one of claims 1 to 7, characterized in that the integral of the function describing the variation of the electric field with time is greater than 1 kV×msec/cm.

9. Method according to claim 8, characterized in that this integral is greater than or equal to 5 kV×msec/cm.

10. Method according to one of claims 1 to 9, characterized in that the electrical pulses are chosen from square wave pulses, electric fields generating exponentially decreasing waves, oscillating unipolar waves of limited duration, oscillating bipolar waves of limited duration, or other wave forms.

11. Method according to one of claims 1 to 10, characterized in that the electrical pulses comprise square wave pulses.

12. Method according to one of claims 1 to 11, characterized in that the electrical pulses are applied with electrodes placed either side of the muscle or placed in contact with the skin.

13. Method according to one of claims 1 to 11, characterized in that the electrical pulses are applied with electrodes introduced inside the muscle.

14. Method according to one of claims 1 to 13, characterized in that the nucleic acid is injected into the muscle.

15. Method according to one of claims 1 to 13, characterized in that the nucleic acid is injected by the systemic route.

16. Method according to claim 15, characterized in that the nucleic acid is injected by the intra-arterial or intravenous route.

17. Method according to one of claims 1 to 13, characterized in that the nucleic acid is administered by the topical, cutaneous, oral, vaginal, intranasal, subcutaneous or intraocular route.

18. Method according to one of claims 1 to 17, characterized in that the nucleic acid is present in a composition containing, in addition, pharmaceutically acceptable excipients for the different modes of administration.

19. Composition according to claim 18, suitable for parenteral administration.

20. Method according to one of claims 1 to 19, characterized in that the nucleic acid is a deoxyribonucleic acid.

21. Method according to one of claims 1 to 19, characterized in that the nucleic acid is a ribonucleic acid.

22. Method according to one of claims 1 to 21, characterized in that the nucleic acid is of

synthetic or biosynthetic origin, or extracted from a virus or from a unicellular or pluricellular eukaryotic or prokaryotic organism.

23. Method according to claim 22, characterized in that the nucleic acid administered is combined with all or part of the components of the organism of origin and/or of the synthesis system.

24. Method according to one of claims 1 to 23, characterized in that the nucleic acid encodes an RNA or a protein of interest.

25. Method according to claim 24, characterized in that the RNA is a catalytic or antisense RNA.

26. Method according to claim 24, characterized in that the nucleic acid encodes a protein chosen from enzymes, blood derivatives, hormones, lymphokines, growth factors, trophic factors, angiogenic factors, neurotrophic factors, bone growth factors, haematopoietic factors, coagulation factors, antigens and proteins involved in the metabolism of amino acids, lipids and other essential constituents of the cell.

27. Method according to claim 26, characterized in that the nucleic acid encodes the angiogenic factors VEGF and FGF, the neurotrophic factors BDNF, CNTF, NGF, IGF, GMF, FGF1, NT3, NT5, the Gax protein, insulin for the treatment of diabetes, growth hormone, a cytokine, α-1-antitrypsin,

calcitonin, leptin and the apolipoproteins, the enzymes for the biosynthesis of vitamins, hormones and neuromediators.

28. Method according to claim 24, characterized in that the nucleic acid codes for an antibody, a variable fragment of single-chain antibody (ScFv) or any other antibody fragment possessing recognition capacities for the purposes of immunotherapy, or codes for a soluble receptor, a peptide which is an agonist or antagonist of a receptor or of an adhesion protein, for an artificial, chimeric or truncated protein.

29. Method according to claim 28, characterized in that the nucleic acid encodes an antiidiotype antibody, a soluble fragment of the CD4 receptor or of the TNFα receptor or of the acetylcholine receptor.

30. Method according to one of claims 26 to 29, characterized in that the nucleic acid encodes a precursor of a therapeutic protein.

31. Method according to one of claims 1 to 30, characterized in that the nucleic acid is in the form of a plasmid.

32. Method according to one of claims 1 to 30, characterized in that the nucleic acid contains a gene of large size and/or introns and/or regulatory elements of small or large size.

33. Method according to one of claims 1 to

30, characterized in that the nucleic acid is an episomal DNA or a yeast artificial chromosome or a minichromosome.

34. Method according to one of claims 1 to 33, characterized in that the nucleic acid contains sequences allowing and/or promoting the expression of the transgene in the muscle.

35. Method according to one of claims 1 to 34, characterized in that the acid is combined with any type of vectors or with any combination of vectors which make it possible to improve the transfer of nucleic acid, such as viruses, synthetic or biosynthetic agents, or beads which are propelled or otherwise.

36. Method according to one of claims 1 to 35, characterized in that the muscle is subjected to a treatment intended to improve gene transfer, a treatment of pharmacological nature in the form of a local or systemic application, or an enzymatic, permeabilizing, surgical, mechanical, thermal or physical treatment.

37. Method according to one of claims 1 to 36, characterized in that it makes it possible to cause the muscle to produce an agent at physiological and/or therapeutic doses, either in the muscle cells, or secreted.

38. Method according to one of claims 1 to 37, characterized in that it makes it possible to

modulate the quantity of transgene expressed by modulating the volume of muscle tissue transfected.

39. Method according to claim 38, characterized in that it makes it possible to modulate the volume of muscle tissue transfected by the use of multiple sites of administration.

40. Method according to one of claims 1 to 39, characterized in that it makes it possible to modulate the quantity of transgene expressed by modulating the number, shape, surface and arrangement of the electrodes, and by varying the intensity, the number, the duration, the frequency and the form of the pulses, as well as the quantity and the volume of nucleic acid for administration.

41. Method according to one of claims 1 to 40, characterized in that it makes it possible to control the localization of the tissues transfected by the volume of tissue subjected to the local electrical pulses.

42. Method according to one of claims 1 to 41, characterized in that it allows a return to the initial situation by removal of the transfected tissue area.

43. Nucleic acid and electric field of an intensity between 1 and 800 volts/cm, as combination product for their administration simultaneously, separately or spaced out over time in vivo to the striated muscle and, for gene therapy based on in vivo

electrotransfection into the striated muscle.

44. Combination product according to claim 43, characterized in that the field intensity is between 4 and 400 volts/cm.

45. Combination product according to claim 43, characterized in that the field intensity is between 30 and 300 volts/cm.

46. Combination product according to one of claims 43 to 45, characterized in that the total duration of application of the electric field is greater than 10 milliseconds.

47. Combination product according to one of claims 43 to 46, characterized in that the application, to the muscle, of the electric field comprises one or more pulses of regular frequency.

48. Combination product according to claim 47, characterized in that the application, to the muscle, of the electric field comprises between 1 and 100,000 pulses of frequency between 0.1 and 1000 hertz.

49. Combination product according to one of claims 43 to 46, characterized in that the electrical pulses are delivered in an irregular manner relative to each other and in that the function describing the intensity of the electric field as a function of the time for one pulse is variable.

50. Combination product according to one of claims 43 to 49, characterized in that the integral of the function describing the variation of the electric

field with time is greater than 1 kV×msec/cm.

51. Combination product according to claim 50, characterized in that this integral is greater than or equal to 5 kV×msec/cm.

52. Combination product according to one of claims 43 to 51, characterized in that the electrical pulses are chosen from square wave pulses, electric fields generating exponentially decreasing waves, oscillating unipolar waves of limited duration, oscillating bipolar waves of limited duration, or other wave forms.

53. Combination product according to one of claims 43 to 52, characterized in that the electrical pulses comprise square wave pulses.

54. Combination product according to one of claims 43 to 53, characterized in that the electrical pulses are applied with electrodes placed either side of the muscle or placed in contact with the skin.

55. Combination product according to one of claims 43 to 53, characterized in that the electrical pulses are applied with electrodes introduced inside the muscle.

56. Combination product according to one of claims 43 to 55, characterized in that the nucleic acid is injected into the muscle.

57. Combination product according to one of claims 43 to 55, characterized in that the nucleic acid is injected by the systemic route.

58. Combination product according to claim 57, characterized in that the nucleic acid is injected by the intra-arterial or intravenous route.

59. Combination product according to one of claims 43 to 55, characterized in that the nucleic acid is administered by the topical, cutaneous, oral, vaginal, intranasal, subcutaneous or intraocular route.

60. Combination product according to one of claims 43 to 59, characterized in that the nucleic acid is present in a composition containing, in addition, pharmaceutically acceptable excipients for the different modes of administration.

61. Composition according to claim 60, suitable for parenteral administration.

62. Combination product according to one of claims 43 to 61, characterized in that the nucleic acid is a deoxyribonucleic acid.

63. Combination product according to one of claims 43 to 61, characterized in that the nucleic acid is a ribonucleic acid.

64. Combination product according to one of claims 43 to 63, characterized in that the nucleic acid is of synthetic or biosynthetic origin, or extracted from a virus or a unicellular or pluricellular eukaryotic or prokaryotic organism.

65. Combination product according to claim 64, characterized in that the nucleic acid administered is combined with all or part of the

components of the organism of origin and/or of the synthesis system.

66. Combination product according to one of claims 43 to 65, characterized in that the nucleic acid encodes an RNA or a protein of interest.

67. Combination product according to claim 66, characterized in that the RNA is a catalytic or antisense RNA.

68. Combination product according to claim 66, characterized in that the nucleic acid encodes a protein chosen from enzymes, blood derivatives, hormones, lymphokines, cytokines, growth factors, trophic factors, angiogenic factors, neurotrophic factors, bone growth factors, haematopoietic factors, coagulation factors, antigens and proteins involved in the metabolism of amino acids, lipids and other essential constituents of the cell.

69. Combination product according to claim 68, characterized in that the nucleic acid encodes the angiogenic factors VEGF and FGF, the neurotrophic factors BDNF, CNTF, NGF, IGF, GMF, FGF1, NT3, NT5, the Gax protein, insulin for the treatment of diabetes, growth hormone, α-1-antitrypsin, calcitonin, leptin and the apolipoproteins, the enzymes for the biosynthesis of vitamins, hormones and neuromediators.

70. Combination product according to claim 66, characterized in that the nucleic acid codes for an antibody, a variable fragment of single-chain

antibody (ScFv) or any other antibody fragment possessing recognition capacities for the purposes of immunotherapy, or codes for a soluble receptor, a peptide which is an agonist or antagonist of a receptor or of an adhesion protein, for an artificial, chimeric or truncated protein.

71. Combination product according to claims 70, characterized in that the nucleic acid encodes an antiidiotype antibody, a soluble fragment of the CD4 receptor or of the TNFα receptor or of the acetylcholine receptor.

72. Combination product according to one of claims 68 to 71, characterized in that the nucleic acid encodes a precursor of a therapeutic protein.

73. Combination product according to one of claims 43 to 72, characterized in that the nucleic acid is in the form of a plasmid.

74. Combination product according to one of claims 43 to 72, characterized in that the nucleic acid contains a gene of large size and/or introns and/or regulatory elements of small or large size.

75. Combination product according to one of claims 43 to 72, characterized in that the nucleic acid is an episomal DNA or a yeast or bacterial artificial chromosome or a minichromosome.

76. Combination product according to one of claims 43 to 75, characterized in that the nucleic acid contains sequences allowing and/or promoting the

expression of the transgene in the muscle.

77. Combination product according to one of claims 43 to 76, characterized in that the acid is combined with any type of vectors or with any combination of vectors which make it possible to improve the transfer of nucleic acid, such as viruses, synthetic or biosynthetic agents, or beads which are propelled or otherwise.

78. Combination product according to one of claims 43 to 77, characterized in that the muscle is subjected to a treatment intended to improve gene transfer, a treatment of pharmacological nature in the form of a local or systemic application, or an enzymatic, permeabilizing, surgical, mechanical, thermal or physical treatment.

79. Combination product according to one of claims 43 to 78, characterized in that it makes it possible to cause the muscle to produce an agent at physiological and/or therapeutic doses, either in the muscle cells, or secreted.

80. Combination product according to one of claims 43 to 78, characterized in that it makes it possible to modulate the quantity of transgene expressed by modulating the volume of muscle tissue transfected.

81. Combination product according to claim 80, characterized in that it makes it possible to modulate the volume of muscle tissue transfected by the

use of multiple sites of administration.

82. Combination product according to one of claims 43 to 81, characterized in that it makes it possible to modulate the quantity of transgene expressed by modulating the number, shape, surface and arrangement of the electrodes, and by varying the field intensity, the number, the duration, the frequency and the form of the pulses, as well as the quantity and the volume of nucleic acid for administration.

83. Combination product according to one of claims 43 to 82, characterized in that it makes it possible to control the localization of the tissues transfected by the volume of tissue subjected to the local electrical pulses.

84. Combination product according to one of claims 43 to 83, characterized in that it allows a return to the initial situation by removal of the transfected tissue area.